Inhibitory Activity of *Allium chinense G. Don*. Extracts to Prodigiosin Synthesis by *Serratia Marcescens*

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Abstract: *Bawang Batak* (*Allium chinense* G. Don.) is one of native medicinal plants utilized as spices in North Sumatera, Indonesia. The plant is known to exhibit antimicrobial activities against several bacterial pathogens. Prospect of finding a new mechanism in combating bacterial infection through quorum-sensing inhibition is an alternative way yet promising strategy to the overuse of antibiotics. The aim of this study is to obtain the optimum concentration of methanolic (MeOH) and ethyl acetate (EtOAc) extracts of *A. chinense* as quorum-sensing inhibitors to prodigiosin synthesis by *S. marcescens.* The inhibition of prodigiosin synthesis is observed visually and measured in absorbance value (A_{534}) compared to control. The results showed that both extracts did not inhibit the growth of tested strain based on optical density (OD_{600}) among tested concentrations. The higher the concentration of MeOH and EtOAc extracts, the less synthesis of prodigiosin by *S. marcescens* in the concentration of 0.3% (w/v) at the end of incubation period (30h). The results showed that MeOH and EtOAc extract may be studied thoroughly for its possibility as quorum-sensing inhibitor following further parameters in the future.

1 INTRODUCTION

Bawang Batak (*Allium chinense* G. Don.) is one of native plant commonly cultivated by the Bataknese in North Sumatera. Members of *Allium*, have also been known as plant material in ethnobotanical medicine. *Allium chinense* is distinct from *kucai* (*Allium tuberosum*), both are commonly utilized as food spices and medicines. *Allium* phytochemical compounds have been reported to possess antimicrobial activity to bacteria, fungi, viruses and parasites (Kyung, 2012). Numerous antimicrobial compounds have been identified, furan (Zanatta e*t al.*, 2007), furfural (Sutar *et al.*, 2012; Chai *et al.*, 2013), and allyl-acetone, allicin, diallyl-disulphide, ajoene, and 3 (Allyl-trisulfanyl)-2-amino propanoic acid (Bah *et al.*, 2012). *Allium chinense* contained majority of phytochemical groups of saponins, flavonoids, terpenoids and steroids (Aulia, 2008). The antimicrobial activity of bulb extract were potential against *Escherica coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* (Naibaho *et al.*, 2015). In addition, bioprospective study of the extracts as antibacterial and antifungal activities have been intensively studied for its application as food preservatives and therapeutic agents against majority infection caused by pathogenic microbes (Benkeblia dan Lanzotti, 2007).

Serratia marcescens is an opportunistic bacterial pathogen with adaptive ability to withstand biocidal properties from chemotherapy, immunotherapy through resistance mechanism. Prodigiosin is a red-pigmented compound synthesized by the species, known as secondary metabolites from tri-pyrrole family with prospect use as multifunctional antibiotics, both as antibacteria and antifungi. Pathogenicity of *S.marcescens* include pneumoniae, urinary tract infection and bacteremia in compromised host (Setiawan *et al.*, 2017). The 16s rRNA region of *S. marcescens* have been sequenced and revealed that quorum sensing regulates the overall pathogenicity of bacteria along with ability to form biofilm and swarming mobility due to serrawetin surfactant (Givskov *et al.*, 1996 ; Givskov *et al.*, 1999). In addition, the species also resistant endogenously against antibiotics like colistin and cephalothin (Matshumura *et al.*, 1998). The use of antibiotic or plant antimicrobials to prevent food spoilage and particular diseases have been practiced

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and leading to end of antibiotic use due to antibiotic resistances (Darshanee *et al*., 2011). Molecular approach that currently gaining popularity is quorum sensing-based inhibition or so called *Quorum Sensing Inhibitor* (QSI) which directly inhibit the virulence factor of a pathogen (Bai dan Rai, 2011). The genetic expression during quorum sensing may be hindered with further consequence of a non-antibiotic resistance occurred (Dong *et al*., 2007; Defoirdt *et al*., 2004). The underlying mechanism of QSI is based on interruption of chemical communication among intraspecific bacteria to conduct quorum sensing hence disabling their phenotypes as whole multi-species embodiment of biofilm yet helping immune or antimicrobial compounds to react more effective towards pathogen (Hentzher dan Givskov, 2003, Nagy, 2010). In this study, we reported an evaluation of bulb extract of *A.chinense* as prospecive QSI phytochemicals based on its performance towards prodigiosin synthesis by S*erratia marcescens*.

2 RESEARCH METHODOLOGY

2.1 Inoculum preparation

Isolate of *S.marcescens* is firstly sub-cultured for 24h in Luria Bertani agar prior to laboratory test. Isolate was collection of Department of Microbiology, University of Sumatera Utara.

2.2 Phytochemical extraction

Bulbs of *Bawang Batak* (*Allium chinense*) were obtained from vegetable garden in Sidikalang, North Sumatera, Indonesia. Bulbs were separated from foliars and roots then sliced to ± 5 mm thickness, and then dried under aeration for 5 d until constant weight. The dried bulbs were then mashed using a blender and filtered to powder (Naibaho *et al.*,2015). A 700 g simplisia powder was immersed into Methanol/ MeOH 75% (v/v) as polar fraction and Ethyl Acetate/ EtOAc 50% (v/v) as semi polar fraction of distilled water. Each samples were macerated for 3 d using a rotaryshaker. Macerates were filtered and concentrated using a rotary evaporator (Büchi® Rotavapor R-200, Sigma-Aldrich). Both concentrated fractions were diluted using Dimethyl sulfoxide (DMSO) for various concentration stocks (Bai and Vittal, 2014).

2.3 Determination of QSI activity

Serratia marcescens is known to produce red pigments namely prodigiosin in growth medium as an indication of quorum sensing occurrence. The measurement of prodigiosin concentration using a spectrophotometer at a wavelength of 534 nm (A_{534}) and the extraction stage refers to Morohoshi et al., (2007). *Serratia marcescens* was grown for 15 hr on fresh Luria-Bertani medium (1%). Production of prodigiosin was monitored in an interval of 5 hr for 30 hr with or without the addition of bulb extracts with concentration variants of 0.02, 0.1, 0.2 and 0.3%. Prodigiosin was extracted from cells in acidified ethanol solution (4% 1 M HCL in ethanol). Prodigiosin production was determined by determining the absorbance ratio extracted at 534 nm. Percentage of prodigiosin inhibition is calculated using following formula with a control value of 100%:

$$\%\text{inhibition} = \frac{\text{Absorbance of control-absorbance of treatment}}{\text{Absorbance of control}} \times 100\%:$$

3 RESULTS AND DISCUSSIONS

Confirmation of QSI activity is based on none inhibition towards growth of reference strain, *Serratia* marcescens. Our results showed that none of tested extracts inhibit the growth of *S.marcescens* (OD_{600}) until the end of incubation period. Control can be seen to produce higher OD than the samples as shown in Figure (1a and 1b)

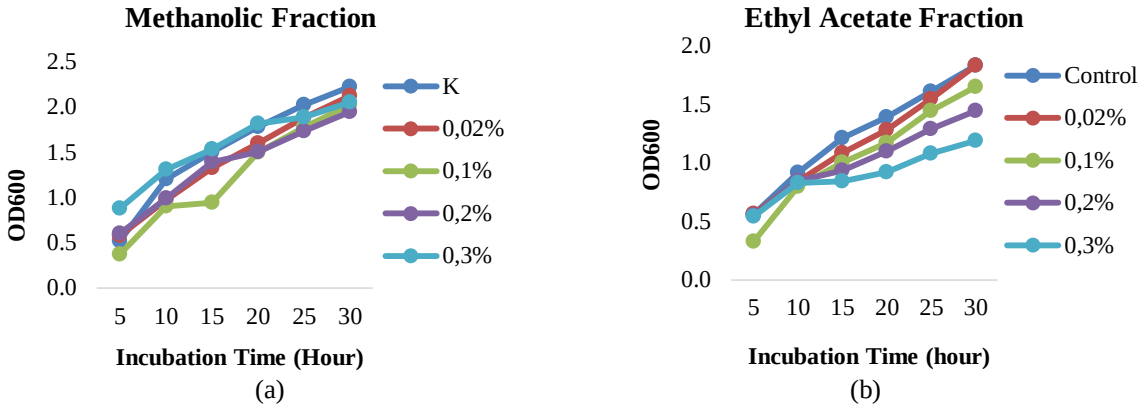


Figure 1: Effect of Bawang Batak MeOH extract at growth of *S. marcescens* (a), . Effect of Bawang Batak EtOAC extract at growth of *S. marcescens* (b).

The results of inhibitory assay using methanolic fractions showed inhibition at concentrations of 0.2 and 0.3%. Prodigiosin production is higher in control than the treatments (Figure 2a). While at the concentration of 0.02 and 0.1%, the production is higher than the control, yet still indicating that there was no inhibition towards prodigiosin at given concentrations. The results from ethyl acetate fraction (EtOAc) was lower than the control (Figure 2B).

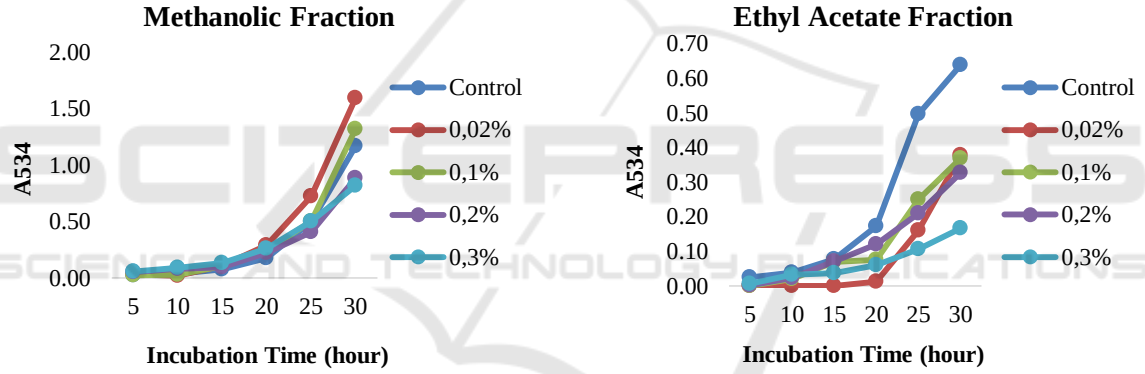
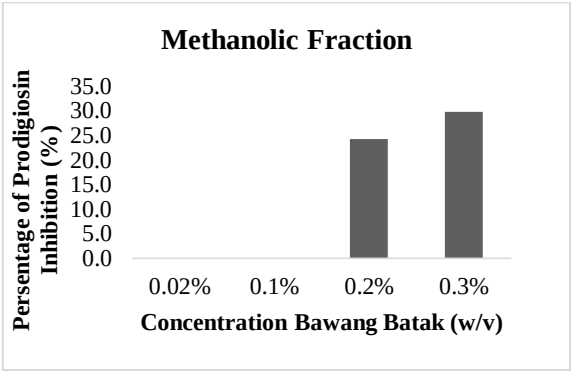
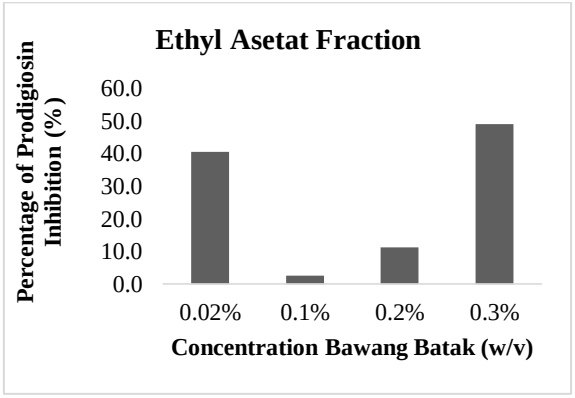


Figure 2: Prodigiosin synthesis ; MeOH extract (a), EtOAC extract (b)

In the end of incubation period (30 hr), methanolic fraction showed a lower prodigiosin production than control at concentration of 0.2 and 0.3% with percentage of inhibition 24.3 and 29.8% which can be seen in Figure 3a. However, from ethyl acetate fraction showed that all tested concentrations inhibit the prodigiosin with the highest observed at concentration of 0.3% with percentage of 49.2% as shown in Figure 3b.



(a)



(b)

Figure 2: Prodigiosin synthesis ; MeOH extract (a), EtOAC extract (b)

Our results showed that bulb extracts of *A.chinense* displayed a potential array of other bioactive properties than previously known as antimicrobials. In this study, MeOH and EtOAc fractions also inhibit the cell signaling communication or quorum sensing. Although phytochemical groups have been identified from these bulb extracts (saponins, triterpenoids, steroids, flavonoids, essential oils), the underlying mechanism in displaying QSI activity is still remain unknown (Liu et al., 2014; Jiang et al., 1999; Kuroda et al., 1995).

In the methanol fraction, the high production of prodigiosin from *Serratia marcescens* is assumed by the effect LB medium used. Luria-Bertani medium is a complex medium that may alter cells metabolic pathway and thus supporting the occurrence of quorum sensing sensing system. In a study using *P. aeruginosa*, it has been shown that increased levels of nutrients may induce the growth of bacterial pigments (Saver et al., 2004). Luria-Bertani medium is also likely to be a complex medium containing signals or other factors, such as surfactants that are needed for swarming and biofilm formation (Holden et al., 1999). However, we are still able to document particular inhibitory activities of extracts in certain tested concentrations as shown in previous figures. Both fractions showed the highest inhibitory value at 0.3% concentration to 30th (incubation period). This is consistent with previous studies stating that, the higher the concentration of extracts, the higher the inhibition (Bai and Vittal., 2014; Packtiavathy et al., 2014). In order to reveal the mechanism of QSI activity exhibited by *A.chinense* bulb extracts, more efforts are needed to support their use as potential QSI in the future.

4 CONCLUSIONS

The results showed that Batak Onion (*Allium chinense, G. Don.*) bulb extract MeOH and EtOAc fractions were potential quorum sensing inhibitors of *Serratia marcescens* without inhibiting the growth of these bacteria.

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